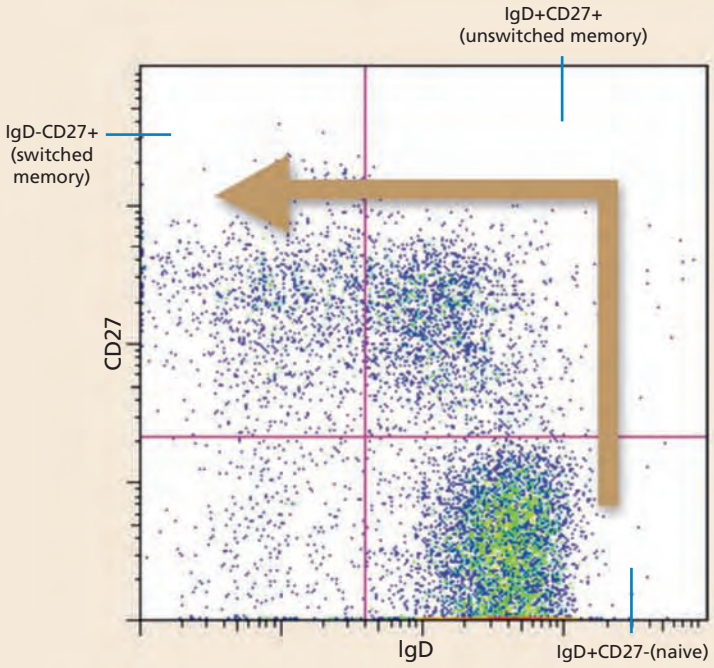
Children's Hospital of Wisconsin®
A member of Children's Hospital and Health System.

MEDICAL COLLEGE OF WISCONSIN

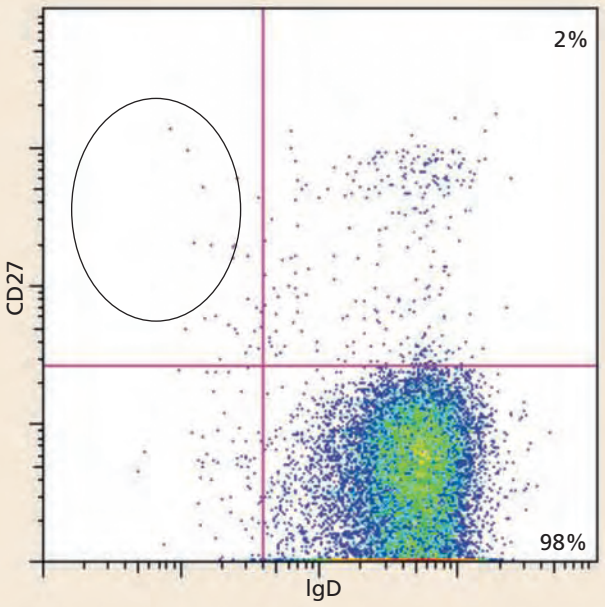
Published through a grant from:
Jeffrey Modell Foundation
Curing PI. Worldwide.

Clinical Flow Cytometry in the Diagnosis of **Primary Immunodeficiencies**

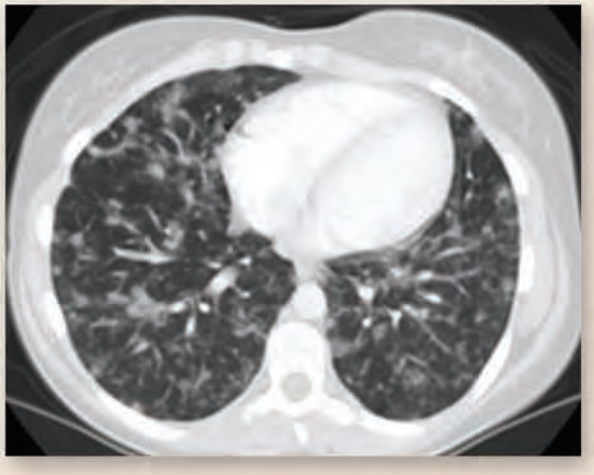
Updated 2011



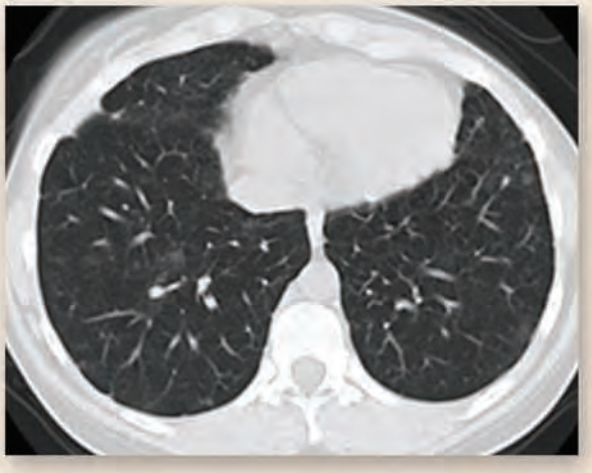
Arrow demonstrates normal B cell maturation.



Abnormal B cell maturation in CVID.



Granulomatous lung disease in a patient with common variable immunodeficiency.



Resolution of lung disease following treatment.

Jeffrey Modell Foundation mission

Vicki and Fred Modell established the Jeffrey Modell Foundation, a 501(c)3 nonprofit organization, in 1987, in memory of their son Jeffrey, who died at the age of fifteen from complications of Primary Immunodeficiency – a genetic condition that is chronic, serious, and often fatal. JMF is a global patient organization devoted to early and precise diagnosis, meaningful treatments, and ultimately, cures through clinical and basic research, physician education, patient support, advocacy, and public awareness. The Foundation has developed a global network of more than 100 Jeffrey Modell Diagnostic and Research Centers worldwide – consisting of 459 expert physicians at 195 academic institutions, in 58 countries and spanning 6 continents – and continues to expand globally.

The Foundation's website, www.info4pi.org, offers resources to patients, families, and healthcare providers.

Table of Contents

Introduction 2

Autoimmune Lymphoproliferative Syndrome 3

Absolute T4 4

Bruton's Tyrosine Kinase 5

Common Variable Immunodeficiency 6

Cytotoxicity/Apoptosis 7

Hyper IgM 8

Neutrophil Oxidative Burst 9

Neutrophil Phagocytosis 10

Perforin – Granzyme 11

Primary Immunodeficiency 1 12

Primary Immunodeficiency 2 13

Severe Combined Immunodeficiency 14

T cell Activation 15

T helper IL17 16

T cell Interleukin Proliferation 17

Toll-like Receptor 18

T cell Mitogen Proliferation 19

T Regulatory – FoxP3 20

X-linked Lymphoproliferatiive Syndrome 21

Test menu summary 22

Clinical Immunodiagnostic and Research Laboratory 24

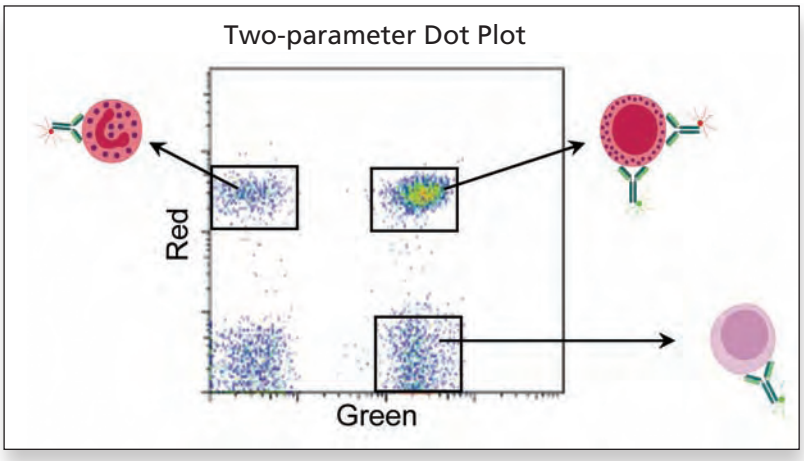
Introduction

The purpose of this booklet is to inform physicians of the commonly used flow cytometric tests for the evaluation of primary immunodeficiency disorders (PIDDs). PIDDs can present at any age and are characterized by recurrent infections, severe infections requiring hospitalization or intravenous antibiotics, and infections caused by opportunistic or unusual organisms. The ability to characterize and define these disorders has improved greatly as our understanding of immunology has progressed.

The evaluation of PIDDs has benefited from the use of flow cytometry. Flow cytometry utilizes antibodies or reagents that emit fluorescence to enumerate the subsets of peripheral blood leukocytes and characterize the functional capacity of these cells. By staining peripheral blood leukocytes with antibodies that are specific for defined antigens, detailed assessments of the different components of the immune system are possible. This educational booklet will provide a brief overview of PIDDs and the specific flow cytometric tests that can be used to diagnose these disorders.

Introduction to flow cytometry

Flow cytometry is a technique in which fluorescently labeled cells flow through a cytometer a single cell at a time. The fluorescent compounds are excited with a laser and detectors measure the light emitted from these compounds. Different fluorescent compounds emit light at different wavelengths, which allows for the discrimination of several different proteins on a cell. When multiple fluorescent compounds are used to analyze a cell population, the results typically are depicted in two-dimensional diagrams. For example, assume a heterogeneous mixture of cells is stained with fluorescently labeled antibodies specific for two proteins, then analyzed on a cytometer with results shown below. A cell that expresses only one protein detected with an antibody (labeled with green fluorescence) will shift along the X-axis but not the Y-axis. A cell that expresses only one protein detected with an antibody (labeled with red fluorescence) will shift along the Y-axis but not the X-axis. A cell expressing both antigens will shift along both the X- and Y-axis. These cells are referred to as "double positive" cells. Cells that do not express either antigen will not shift along either the X- or Y-axis, which can be seen as a population in the lower left corner. These cells are referred to as "double negative" cells.



Autoimmune Lymphoproliferative Syndrome

Utility: Diagnostic screen for autoimmune lymphoproliferative syndrome.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

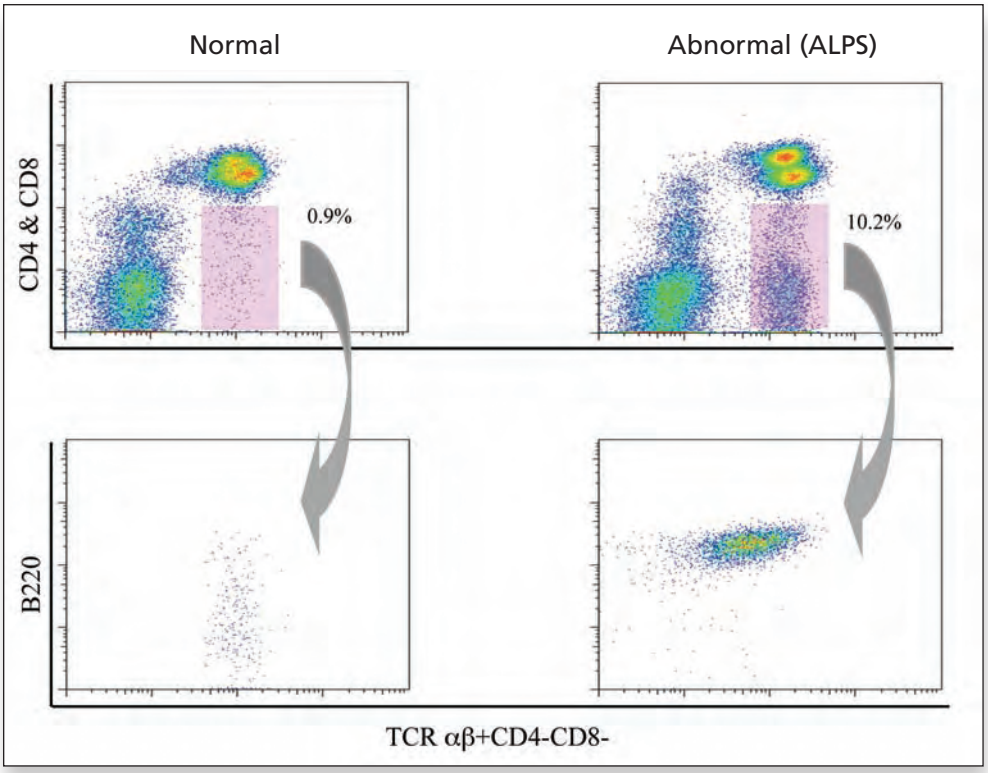
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 11, 88188.

Clinical indication/general description

Autoimmune lymphoproliferative syndrome (ALPS), also known as Canale-Smith syndrome, is caused by a defect in apoptosis (programmed cell death) of lymphocytes via the Fas pathway leading to the abnormal accumulation of lymphocytes. Patients with ALPS present clinically with lymphadenopathy, hepatosplenomegaly and autoimmunity (autoimmune cytopenias and other autoimmune disorders) and have an increased, long-term risk to develop lymphomas.

Detection methodology

Normally, less than 1 percent of T cells that express the T cell receptor alpha and beta chains (TCRαβ+) do not express either the CD4 or the CD8 co-receptors. These T cells are termed double negative T-cells (DNT). In ALPS, the number of TCRαβ+DNT cells is increased. Additionally, the TCRαβ+DNT in ALPS express an isoform of CD45 that usually is expressed only on B cells, the B220 antigen. An increased number of B220+, TCRαβ+DNT cells are found in all characterized forms of ALPS.



Absolute T4

Utility: 1) Monitor treatment efficacy for human immunodeficiency virus (HIV).
2) Establish decision points for antiviral therapeutic initiation.

Specimen: 1 – 4 mL peripheral blood in sodium heparin (green top).

CPT codes: 86359, 86360, 88184, 88185, 88187.

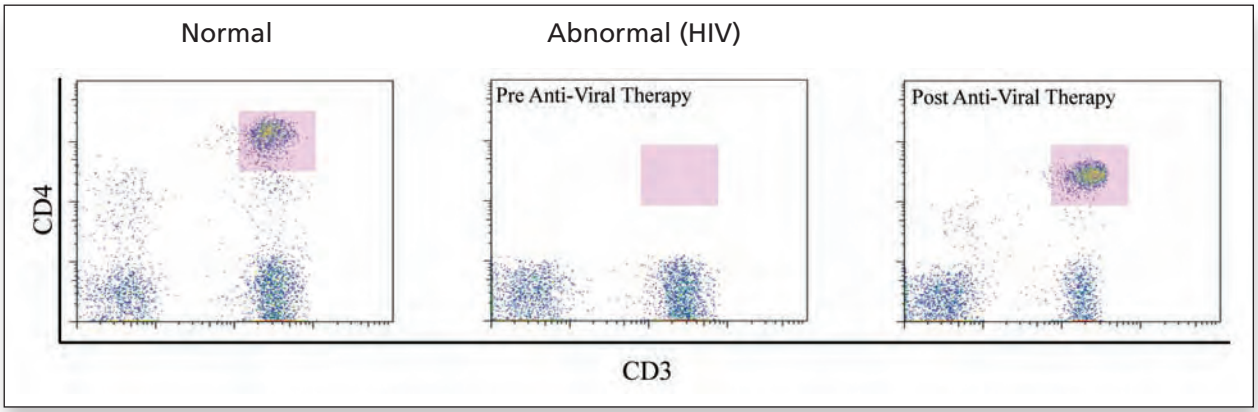
Clinical indication/general description

Human immunodeficiency virus type 1 (HIV-1) infects CD4+ T cells (helper T cells) leading to their premature death. The subsequent decrease in the number of CD4+ T cells results in the Acquired Immunodeficiency Syndrome (AIDS) and an increased susceptibility to opportunistic infections. Monitoring the number of CD4+ T cells is useful to assess the risk of infection and to monitor the response to anti-retroviral therapy.

Detection methodology

Flow cytometric detection of CD4 and CD8 cells is used to determine the absolute CD4 and CD8 counts. AIDS presents with a severe reduction or absence of T helper cells (CD3+CD4+) with an absolute CD4 value < 200cells/ml, a low CD4:CD8 ratio (typically <1.00) and infections.

Note: The CD3 antigen exclusively is expressed on T cells.



Bruton's Tyrosine Kinase

Utility: 1) Diagnostic screen for X-linked agammaglobulinemia (XLA). 2) Detection of carrier status in female relative of XLA. 3) Evaluation of hypogammaglobulinemia in male patients.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

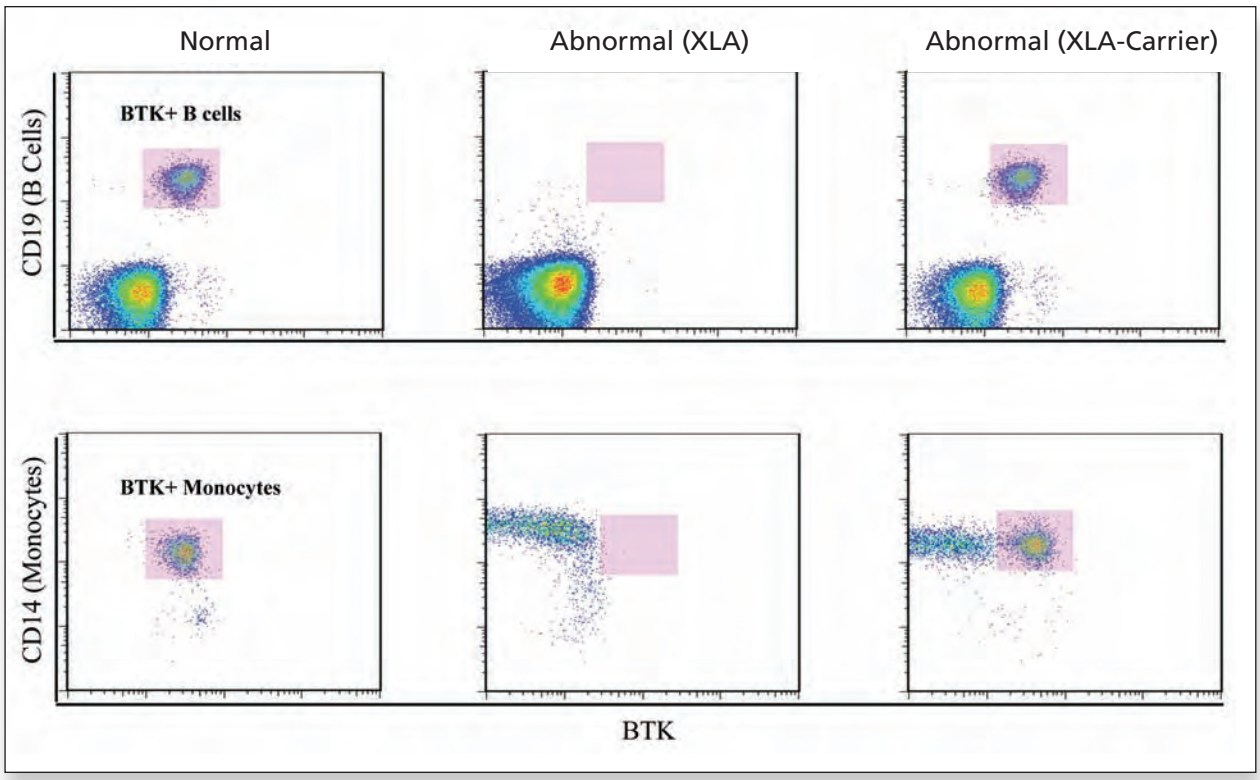
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 5, 88188.

Clinical indication/general description

X-linked agammaglobulinemia (XLA), also known as Bruton's agammaglobulinemia, is characterized by a marked reduction or absence of peripheral blood B cells and profound hypogammaglobulinemia of all isotypes (IgG, IgA, IgM and IgE). Patients with XLA present in early childhood with recurrent infections, in particular with encapsulated bacteria, as well as chronic enteroviral infections. XLA is caused by mutations in the Bruton's Tyrosine Kinase (BTK) gene, which is essential for the development of B cells. Some mutations in BTK result in a milder clinical and laboratory phenotype and are therefore described as leaky.

Detection methodology

XLA presents with severe reduction or absence of B cells (CD19+). Therefore, BTK protein expression is determined in CD14+ monocytes since these cells also express BTK. In XLA, monocytes express either no or very low amounts of BTK protein. Women who carry the mutated allele express normal numbers of B cells that produce normal levels of BTK due to nonrandom X inactivation. However, only 50 percent of monocytes express the BTK protein and this observation can be used to determine carrier status of relatives of affected children.



Common Variable Immunodeficiency

Utility: 1) Diagnostic screen for common variable immunodeficiency (CVID). 2) Assess B cell response to immunotherapeutics. 3) Assess B cell subset reconstitution post-stem cell or bone marrow transplant.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

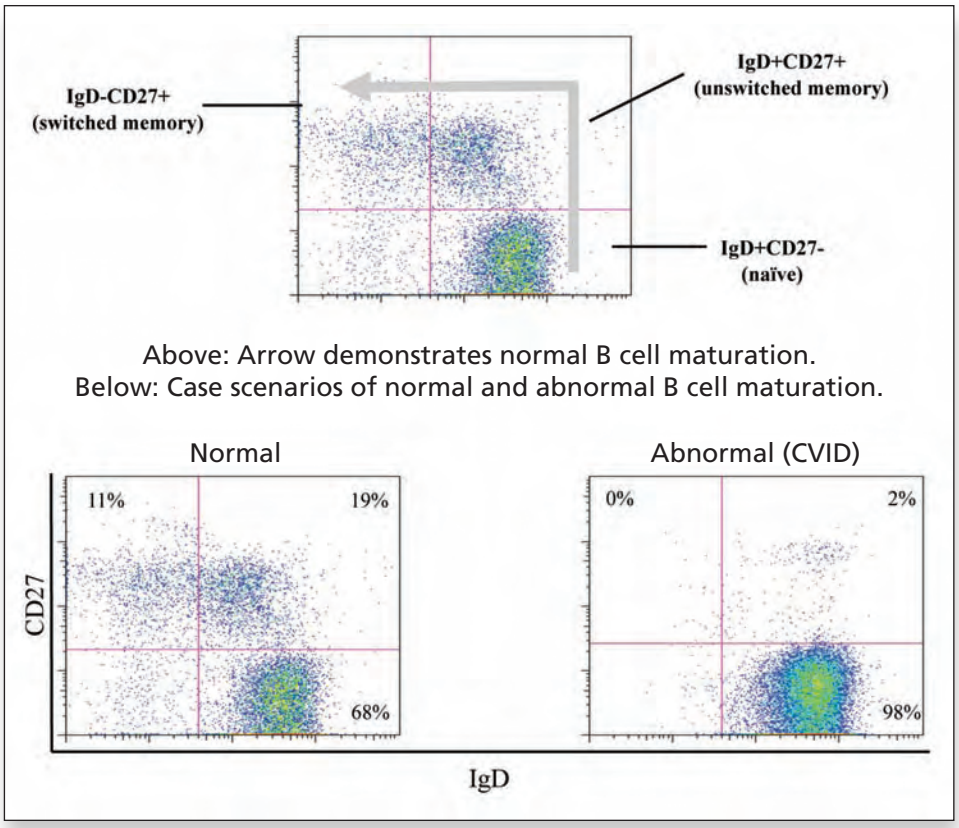
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 10, 88188.

Clinical indication/general description

CVID is characterized by a low serum IgG and either a low IgA, a low IgM or both a low IgA and IgM along with an impaired ability to make specific antibodies in response to immunization. CVID may occur at any age and such patients have recurrent respiratory tract infections in particular with encapsulated bacteria and *Mycoplasma sp.* B cell numbers may be normal or decreased and T cell numbers also may be reduced. Patients with CVID are at increased risk to develop granulomatous or lymphocytic interstitial lung disease (GLILD), autoimmunity (such as autoimmune thrombocytopenic purpura or autoimmune hemolytic anemia) and lymphomas.

Detection methodology

The CVID assay analyzes B cell maturation that occurs in a sequential immunophenotypic pattern (illustrated below). B cells that are naïve (antigen inexperienced) do not express CD27. Upon engagement with antigen, B cells express CD27 and are termed memory B cells. Memory B cells are further classified as unswitched memory B cells (express CD27 and IgD) and switched memory B cells (express CD27 but do not express IgD). Patients with CVID that have a markedly reduced number of switched memory B cells are at increased risk to have a more severe clinical phenotype and to develop GLILD. Low numbers of switched memory B cells also can be seen in other disorders affecting B cell maturation, such as hyper-IgM syndrome.



Above: Arrow demonstrates normal B cell maturation.
Below: Case scenarios of normal and abnormal B cell maturation.

Cytotoxicity/Apoptosis

Utility: 1) Diagnostic screen for hemophagocytic lymphohistiocytosis. 2) Functional evaluation of natural killer (NK) function.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

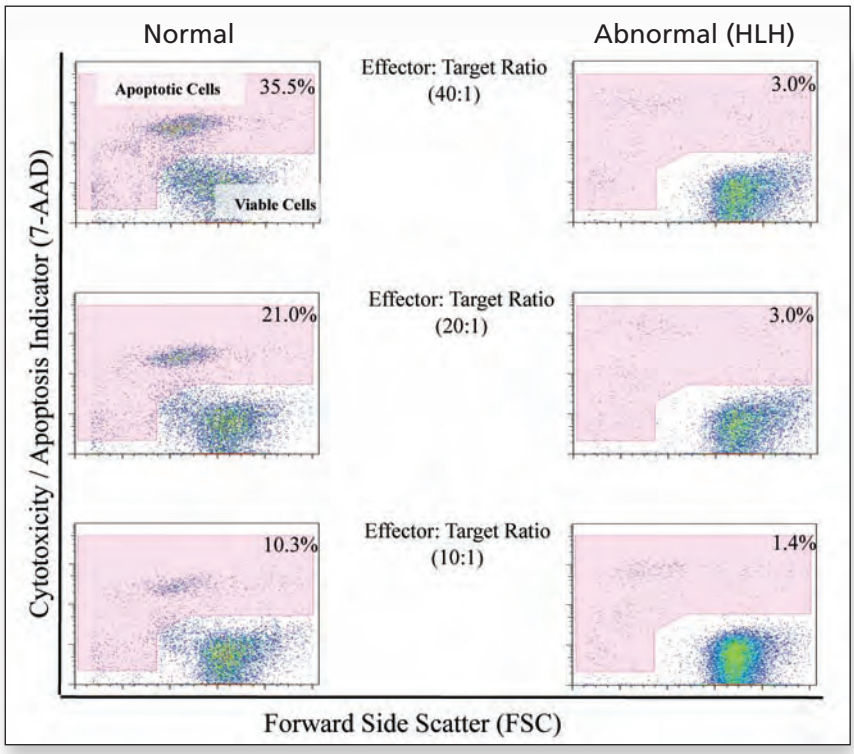
CPT codes: 86849, 88187.

Clinical indication/general description

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening disorder characterized by excessive lymphocytic activation and cytokine secretion, macrophage activation, subsequent hemophagocytosis of blood cells and organ dysfunction. This disorder usually is triggered by viral infections and typically presents at a young age. All known genetic mutations associated with HLH occur in genes encoding proteins required to kill virally infected cells. Perforin, a pore-forming protein stored in cytotoxic granules and secreted by NK cells and cytotoxic CD8+ lymphocytes, is required to kill virally infected and malignant cells. Mutations in perforin or other granule associated proteins cause HLH. Individuals with HLH exhibit defective NK cell function. In addition, defective NK cell function may be seen in patients with recurrent, severe viral infections, particularly infections with herpes viruses.

Detection methodology

A functional flow cytometric-based assay is used to evaluate the ability of a patient's NK cells to induce apoptosis of a target cell population. Target cells (K562) are fluorescently labeled to differentiate them from the patient's peripheral blood mononuclear cells. PBMCs containing NK cells (such as effectors) are cultured with target cells at different effector to target cell ratios. Target cell apoptosis is measured by incorporation of the fluorescent dye 7-AAD, which binds to the DNA in apoptotic cells. The cytotoxicity/apotosis assay will detect defects in NK cell function in patients with clinical symptoms of HLH, and it also can be used to test the function of NK cells in patients with severe or chronic viral infections.



Hyper IgM

Utility: 1) Diagnostic screen for X-linked (CD40L) and autosomal recessive (CD40) hyper IgM syndrome. 2) Detection of carrier status in female relative of X-linked hyper IgM syndrome.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 6, 88187.

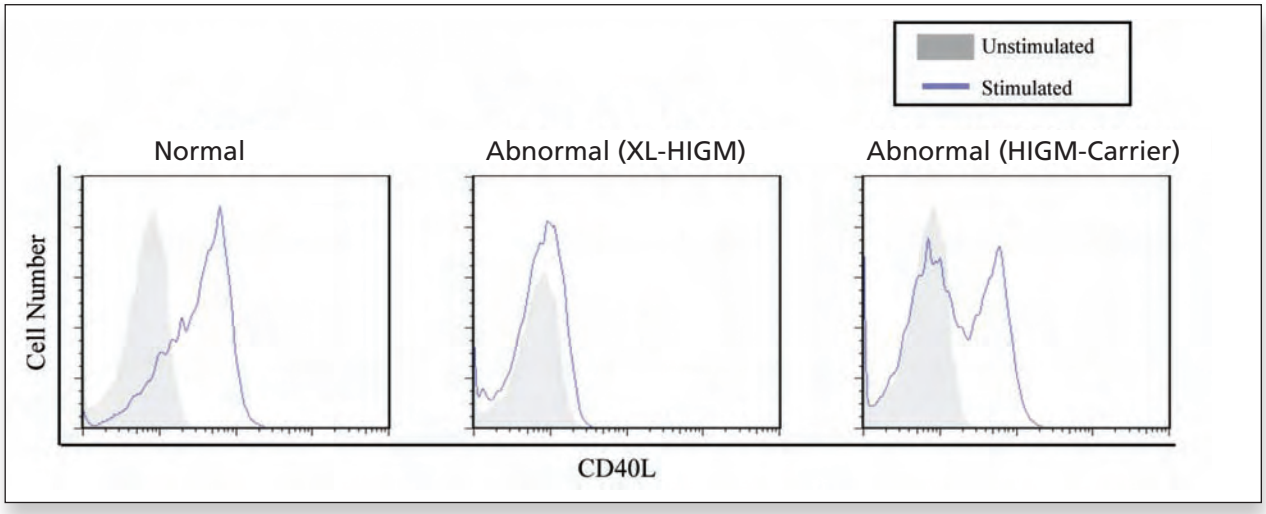
Clinical indication/general description

Hyper-IgM (HIGM) syndrome is a group of primary immunodeficiencies characterized by the absence of immunoglobulin class-switching. Patients with HIGM syndrome exhibit low serum IgG, IgA and IgE levels with normal or elevated serum IgM levels. HIGM syndrome can be caused by mutations in *CD40LG* (Type 1, X-linked HIGM syndrome), *CD40* (Type 3, autosomal recessive HIGM syndrome), activation-induced cytidine deaminase (AID, Type 2, autosomal recessive), or uracil-DNA glycosylase (UNG, Type 5, autosomal recessive). T lymphocytes up-regulate CD40L upon activation that interacts with CD40 on B cells resulting in immunoglobulin class switching, a process that requires AID and UNG. CD40L also interacts with CD40 on monocytes resulting in activation of cell-mediated immune responses. HIGM patients with mutations in *CD40* or *CD40LG* are prone to a variety of bacterial and viral infections as well as opportunistic infections with *Pneumocystis jiroveci* or *Cryptosporidium*. HIGM patients, due to mutations in *AID* or *UNG*, suffer from recurrent infections but do not show susceptibility to opportunistic infections.

Detection methodology

X-linked hyper IgM (XL-HIGM, Type 1) presents with the inability of activated T helper cells (CD3+CD4+) to up-regulate CD40L (CD154) surface expression. Peripheral blood cells are activated pharmacologically and the expression of CD40L is determined by flow cytometry. Female carriers show two populations of CD4 cells: one with CD40L expression and one without expression. This assay also detects CD40 expression on B cells and monocytes (autosomal recessive, Type 3).

Note: This assay does not detect defects in UNG or AID.



Neutrophil Oxidative Burst

Utility: 1) Functional evaluation of neutrophil oxidative burst potential. 2) Diagnostic screen for chronic granulomatous disease (CGD). 3) Detection of carrier status in female relative of CGD patient.

Specimen: 1 – 4 mL peripheral blood in sodium heparin (green top).

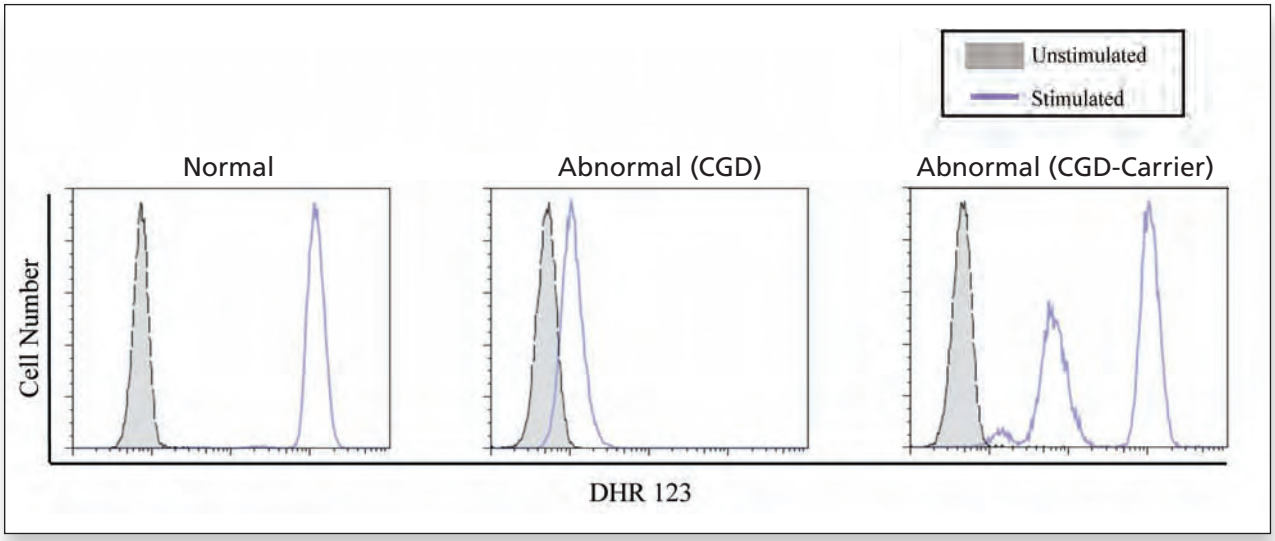
CPT codes: 88184, 88185, 88187.

Clinical indication/general description

CGD is a group of disorders characterized by a defective oxidative burst resulting in an inability to generate toxic oxygen radicals (superoxide) that are required to kill bacteria. Patients affected by this disorder present with recurrent bacterial infections or abscesses, particularly of the skin, subcutaneous areas or regional lymph nodes. In CGD, microbial killing is defective due to mutations in one of four known components of the NADPH oxidase system: one X-linked (gp91-phox) and three autosomal recessive (p22-phox, p47-phox and p67-phox).

Detection methodology

This is a flow cytometric functional assay used to assess the ability of neutrophils to produce an oxidative burst. Neutrophils are loaded with dihydrorhodamine (DHR) dye and then activated with phorbol-12-myristate-13 acetate (PMA). Normal activated neutrophils produce superoxides that oxidize DHR resulting in the emission of fluorescence that is quantitated by flow cytometry. Neutrophils from patients with CGD cannot generate superoxide and therefore do not oxidize DHR.



Neutrophil Phagocytosis

Utility: Functional evaluation of neutrophil phagocytosis.

Specimen: 1 – 4 mL peripheral blood in sodium heparin (green top).

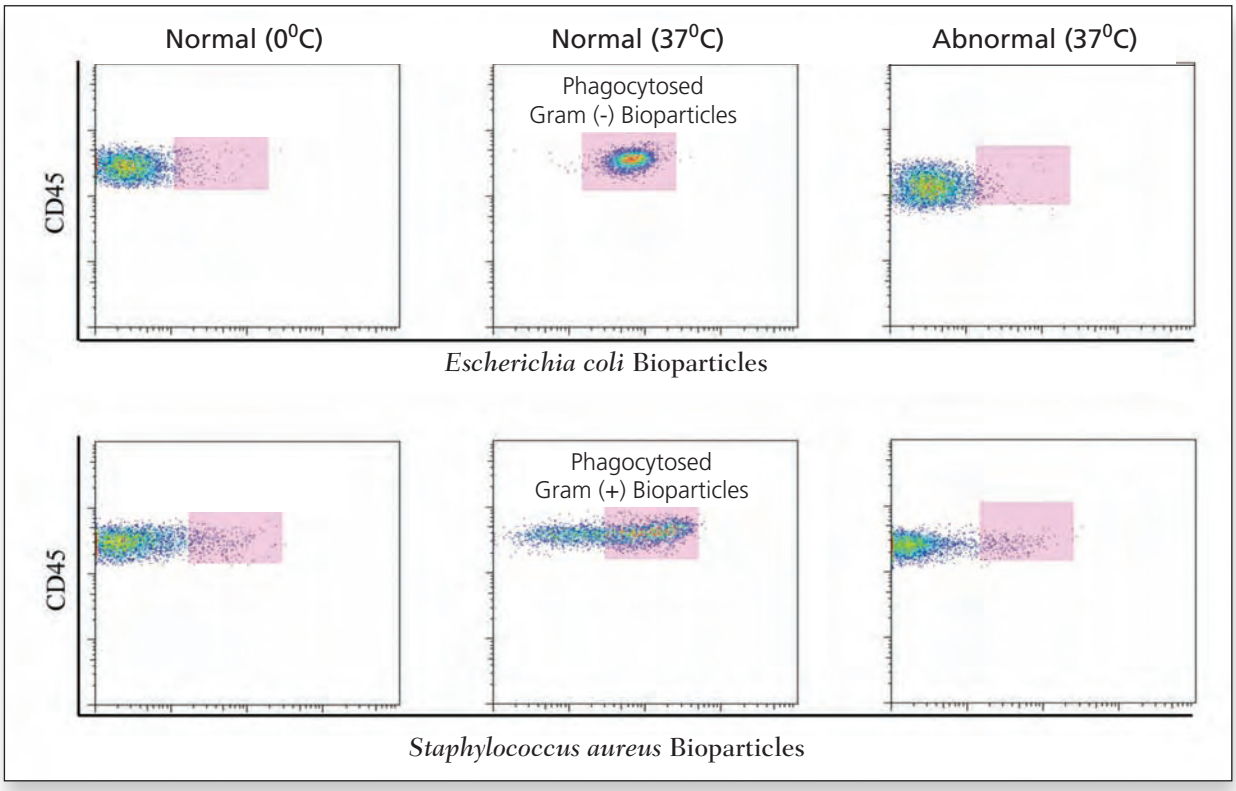
CPT codes: 86344 x 2, 88187.

Clinical indication/general description

Primary neutrophil disorders are associated with recurrent bacterial infections, abnormal pus formation and poor wound healing. Neutrophils must migrate through tissues and phagocytose the invading organisms, a process that requires actin polymerization. A variety of disorders may be associated with the inability of neutrophils to migrate and phagocytose bacteria, such as neutrophil actin dysfunction disorder and Wiskott-Aldrich syndrome. In addition, a variety of drugs and other extrinsic factors can affect the phagocytic function of neutrophils.

Detection methodology

A flow cytometric-based assay is used to measure the phagocytosis of bio-particles coated with *E. coli* or *S. aureus* by neutrophils. The particles are conjugated to pHrodo, a novel fluorescent dye that dramatically increases in fluorescence when internalized within the neutrophil. After incubation of the bioparticles with the patient's neutrophils, emission of fluorescence indicates normal phagocytosis.



Perforin – Granzyme

Utility: 1) Diagnostic screen for hemophagocytic lymphohistiocytosis (HLH). 2) Determination of intracellular perforin, granzyme A and granzyme B within cytolytic lymphocytes.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

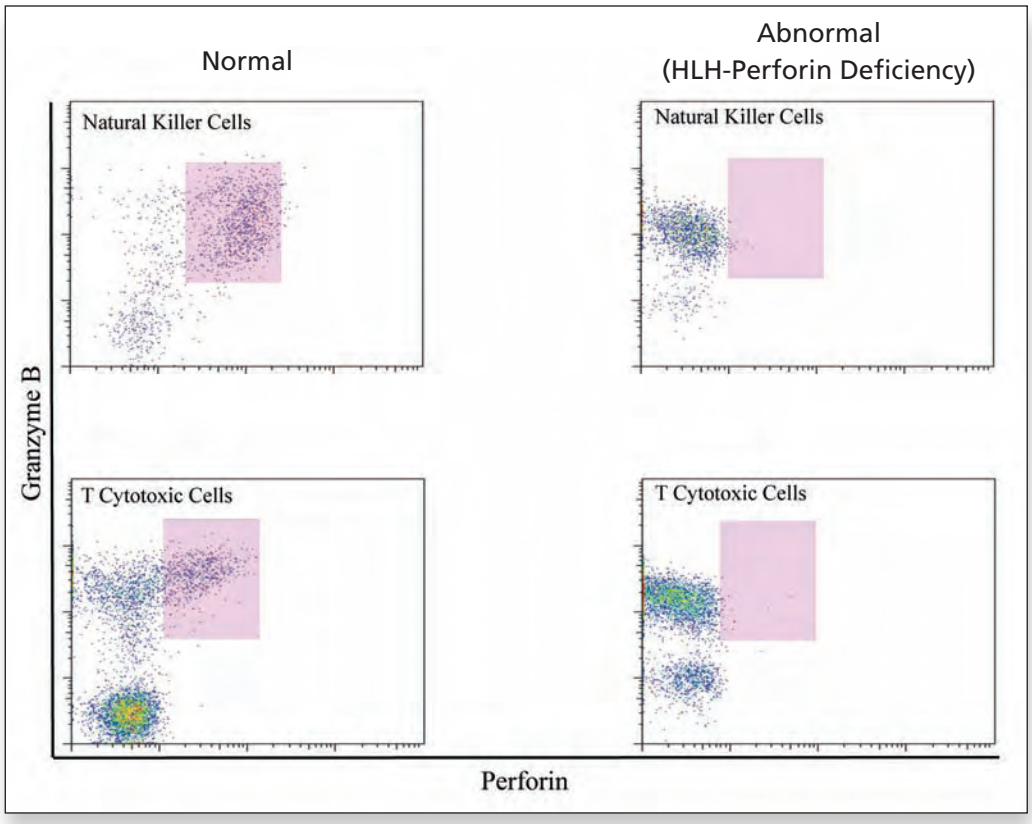
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 5, 88187.

Clinical indication/general description

HLH is a rare, life-threatening disorder characterized by excessive lymphocytic activation and cytokine secretion, macrophage activation, subsequent hemophagocytosis of blood cells and organ dysfunction. This disorder usually is triggered by viral infections and typically presents at a young age. All known genetic mutations associated with HLH occur in genes encoding proteins in natural killer (NK) cells and cytotoxic lymphocytes that are required to kill virally infected cells. Perforin, a pore-forming protein stored in cytotoxic granules and secreted by NK cells and cytotoxic lymphocytes, is required to kill virally infected and malignant cells. Mutations in perforin, as well as other granule-associated proteins, have been shown to cause HLH. Individuals with HLH also exhibit defective NK cell function.

Detection methodology

The assay is designed to enumerate the percentage of NK cells (CD56+CD8-) and T cytotoxic cells (CD56-CD8+) expressing perforin, granzyme A and granzyme B. This assay can detect patients with HLH that lack perforin, and also evaluates the expression of cytotoxic molecules (perforin, granzyme A and granzyme B) in NK cells of patients with defective NK cell cytotoxic function.



Primary Immunodeficiency 1

Utility: General evaluation for T, B and natural killer (NK) cell populations.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 4, 88187.

Clinical indication/general description

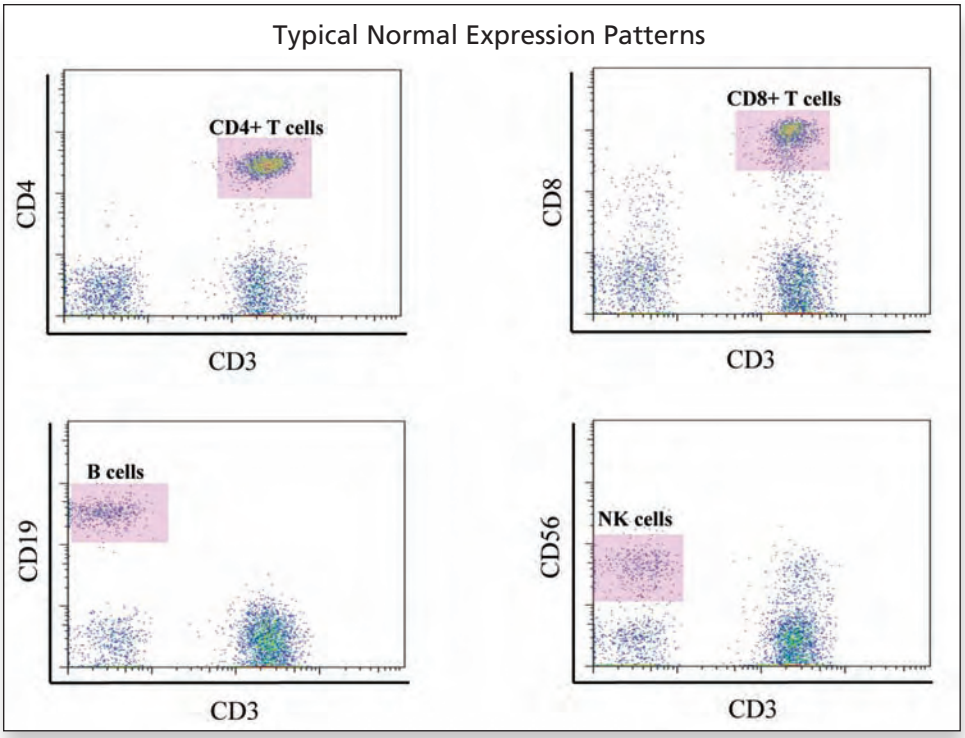
Primary immunodeficiencies (PIDDs) and secondary immunodeficiencies present with recurrent upper and lower respiratory tract infections (encapsulated and atypical bacteria), deep-seated infections, recurrent or deep-seated abscesses, intractable diarrhea and failure to thrive. In addition, PIDDs also can present with autoimmune manifestations and malignancies. Evaluation of patients with these manifestations includes enumeration of the different types of lymphocytes (T cells, B cells and NK cells) because an absolute lymphocyte count from a complete blood count differential can miss important deficiencies in specific subsets of lymphocytes.

The PID1 assay enumerates the numbers of helper (CD4) and cytotoxic (CD8) T cells, B cells and NK cells. Numerous immunodeficiencies associated with decreased numbers of T cells, B cells or NK cells can be detected with this assay including DiGeorge syndrome (low T cell numbers will be detected), AIDS (low CD4 cell counts will be detected), X-linked agammaglobulinemia (low B cells will be detected) and NK cell deficiencies (low NK cell numbers will be detected).

Detection methodology

The assay is designed to enumerate the percent and absolute cell counts of T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+/CD56+).

Note: This assay does not detect neutrophil adhesion defects (leukocyte adhesion deficiency) or signs of immune activation.



Primary Immunodeficiency 2

Utility: 1) Comprehensive initial screening for cellular and humoral inherited immune deficiency. 2) Leukocyte adhesion deficiencies.

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 13, 88188.

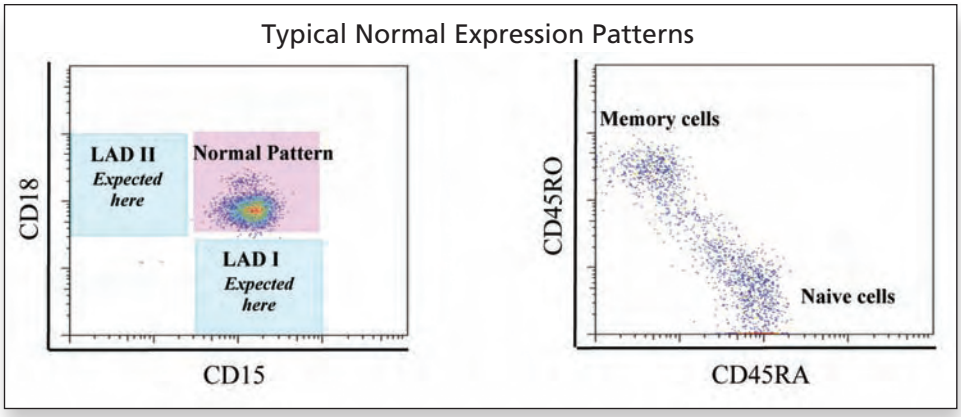
Clinical indication/general description

Primary immunodeficiencies (PIDDs) and secondary immunodeficiencies present with recurrent upper and lower respiratory tract infections (encapsulated and atypical bacteria), deep-seated infections, recurrent or deep-seated abscesses, intractable diarrhea and failure to thrive. In addition, PIDDs also can present with autoimmune manifestations and malignancies. Evaluation of patients with these manifestations includes enumeration of the different types of lymphocytes (T cells, B cells and natural killer (NK) cells) because an absolute lymphocyte count from a complete blood count differential can miss important deficiencies in specific subsets of lymphocytes.

The PID2 assay is a more extensive enumeration of the different subsets of lymphocytes and also will identify patients with defects in the expression of adhesion molecules on neutrophils. Leukocyte adhesion deficiency Type I (LAD-I), also known as LFA-1 deficiency, is caused by a decreased expression of CD18 and LAD-II is caused by the decreased expression of CD15. Additionally, the PID2 assay also will detect the increased activation of CD4 T cells or CD8 T cells that may be caused by an ongoing viral infection or immune dysregulation. Activated human T cells express the HLA class II protein (CD4+DR+ or CD8+DR+) and an isoform of the CD45 antigen known as CD45RO. Numerous immunodeficiencies associated with decreased numbers of T cells, B cells or NK cells can be detected with this assay including DiGeorge syndrome (low T cell numbers will be detected), AIDS (low CD4 cell counts will be detected), X-linked agammaglobulinemia (low B cells will be detected) and NK cell deficiencies (low NK cell numbers will be detected).

Detection methodology

The assay is designed to enumerate the percent and absolute cell counts of T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+/CD56+). The assay examines the expression of CD18 and CD15 on neutrophils, and enumerates the numbers of T cells that are activated (CD4+HLADR+ or CD8+HLADR+ cells and CD4+CD45RO+ cells). CD4+CD45RO+ cells also are referred to as "memory" T cells.



Severe Combined Immunodeficiency

Utility: Confirmatory test of severe combined immunodeficiency (SCID) newborn screening.

Specimen: 1 – 2 mL peripheral blood in sodium heparin (green top).

CPT codes: 86359, 86360, 86355, 86357, 88184, 88185, 81887.

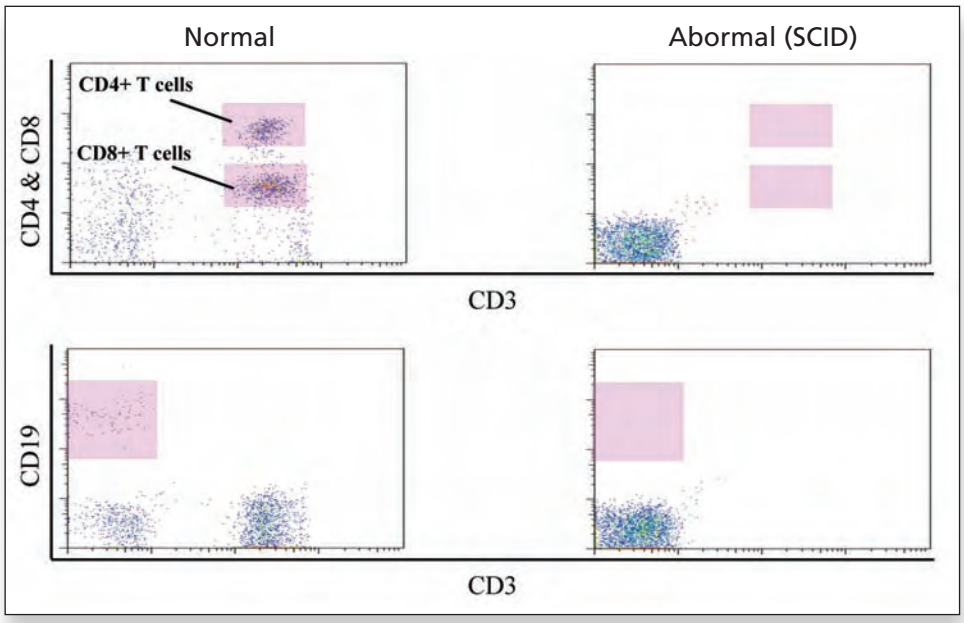
Clinical indication/general description

SCID is characterized by profound impairment of both cellular and humoral immunity due to the absence or markedly diminished number of T cells and variably decreased numbers of B cells or natural killer (NK) cells. Infants affected with SCID typically present within the first year of life with recurrent bacterial and viral infections, opportunistic infections (such as pneumocystis pneumonia), and can have fatal responses to live viral vaccination. SCID is life threatening within the first year of life if not detected and treated with hematopoietic stem cell transplantation. This is a limited assay that only enumerates the numbers of CD4+ T cells, CD8+ T cells, B cells and NK cells. This assay is designed as a confirmatory immunophenotyping for infants who fail the newborn screening program for SCID in the state of Wisconsin.

Detection methodology

The assay is designed to enumerate the percent and absolute cell counts of T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+/CD56+). This assay also enumerates the percentage of naïve CD4+ and CD8+ T cells.

Note: The assay should only be ordered for confirmatory immunophenotyping as part of the state of Wisconsin's newborn screening program for SCID.



T cell Activation

Utility: Functional evaluation of T cell activation.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

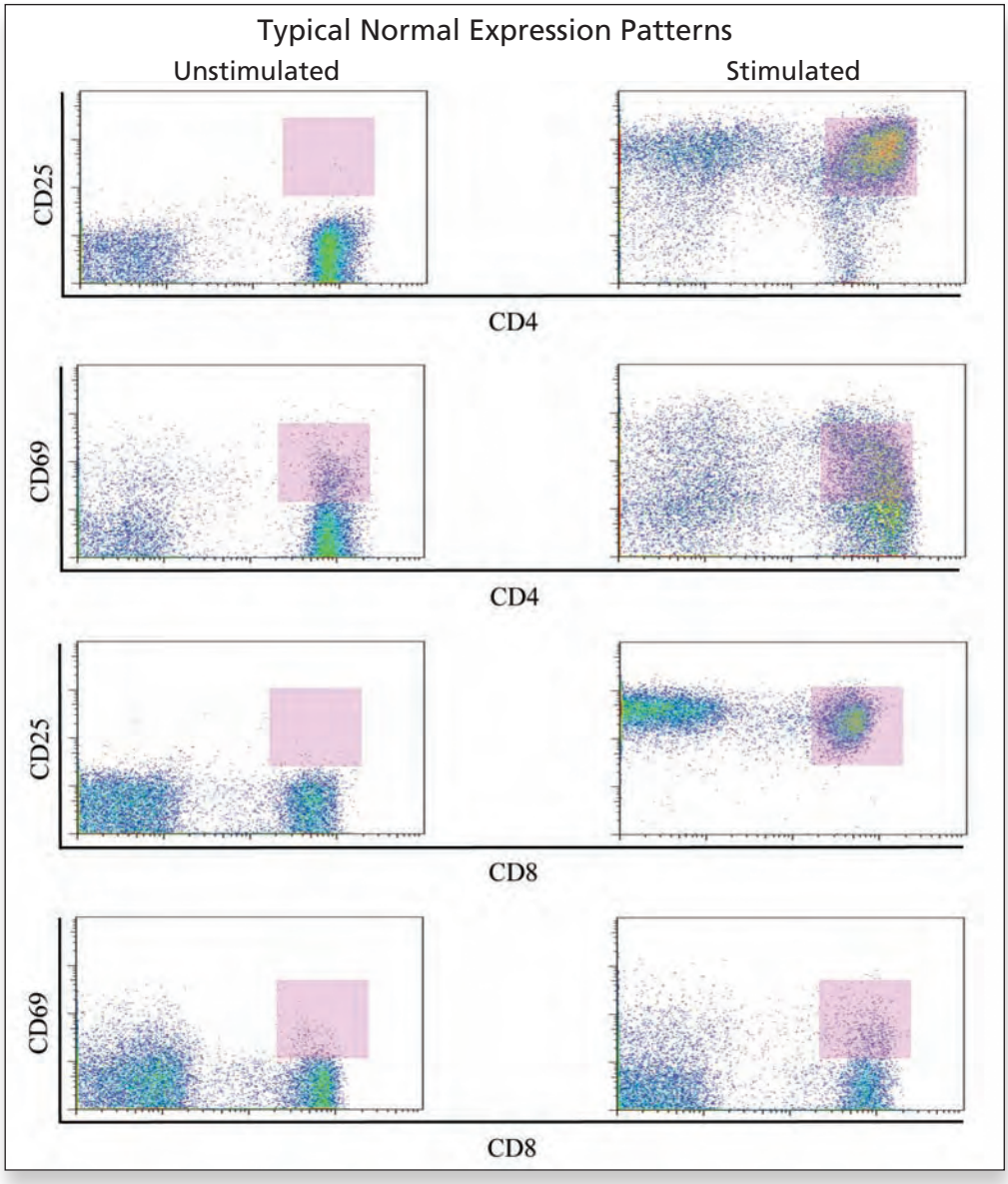
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 6, 81887.

Clinical indication/general description

When T cells are activated through the T cell receptor, a number of signal transduction events occur leading to the expression of the surface marker CD69 at early time points (4 hours) and CD25 at late time points (4 days). This assay can be used to evaluate infants with defective T cell function.

Detection methodology

A functional flow cytometric-based assay evaluates activation of T helper cells (CD3+CD4+) and T cytotoxic cells (CD3+CD8+) using CD25 and CD69 surface expression. Quiescent lymphocytes express low levels of the CD25 and CD69 antigens and upon activation the expression of CD25 and CD69 is markedly increased.



T helper IL17

Utility: Diagnostic screen for hyper – immunoglobulin E syndrome (HIES).

Specimen: 4 – 10 mL peripheral blood in sodium heparin (green top).

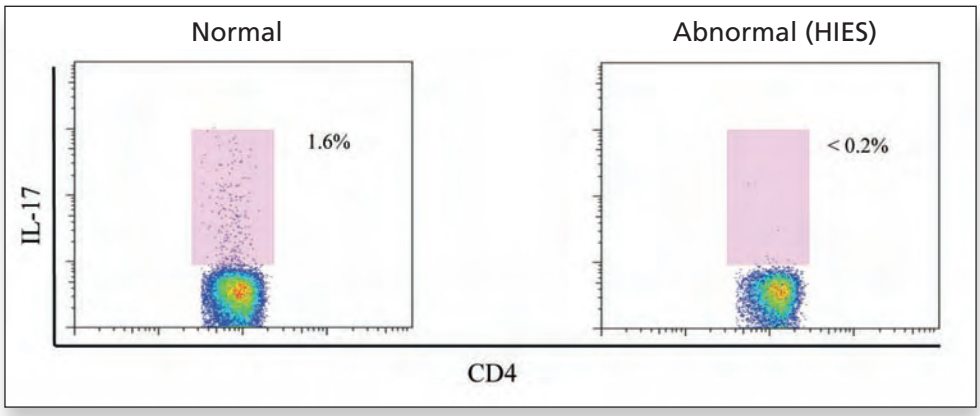
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 6, 81887.

Clinical indication/general description

HIES, also known as Job's syndrome, is characterized by pulmonary infections, staphylococcal abscesses, eczema and abnormalities of bone and connective tissue. IgE levels typically are very high. HIES syndrome can look very similar to severe eczema, thus a laboratory test to differentiate these syndromes is clinically useful. The defects in Hyper-IgE syndrome are caused by mutations in the transcription factor *STAT3*. STAT3 is required to induce CD4+ T cells to produce IL-17, a cytokine that is important for the elicitation of an effective immune response to several bacteria and fungi. The T helper IL-17 funtional assay measures the ability of CD4+ T cells to make IL-17, which is defective in patients with HIES.

Detection methodology

Peripheral blood mononuclear cells (PBMCs) are activated *in vitro* with PMA and ionomycin to induce the expression of IL-17 in normal T helper cells (CD3+CD4+), which is measured by flow cytometry using antibody that specifically recognizes IL-17. Simultaneously, IFN-*y* is measured within the T cytotoxic cell (CD3+CD8+) as a control to ensure adequate activation of T cells. An extremely low percentage of IL-17+ CD4+ T cells is associated with HIES, whereas the percentage of IL-17+ CD4+ cells is normal or slightly reduced in eczema.



T cell Interleukin Proliferation

Utility: Functional evaluation of T cell proliferation to interleukins.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

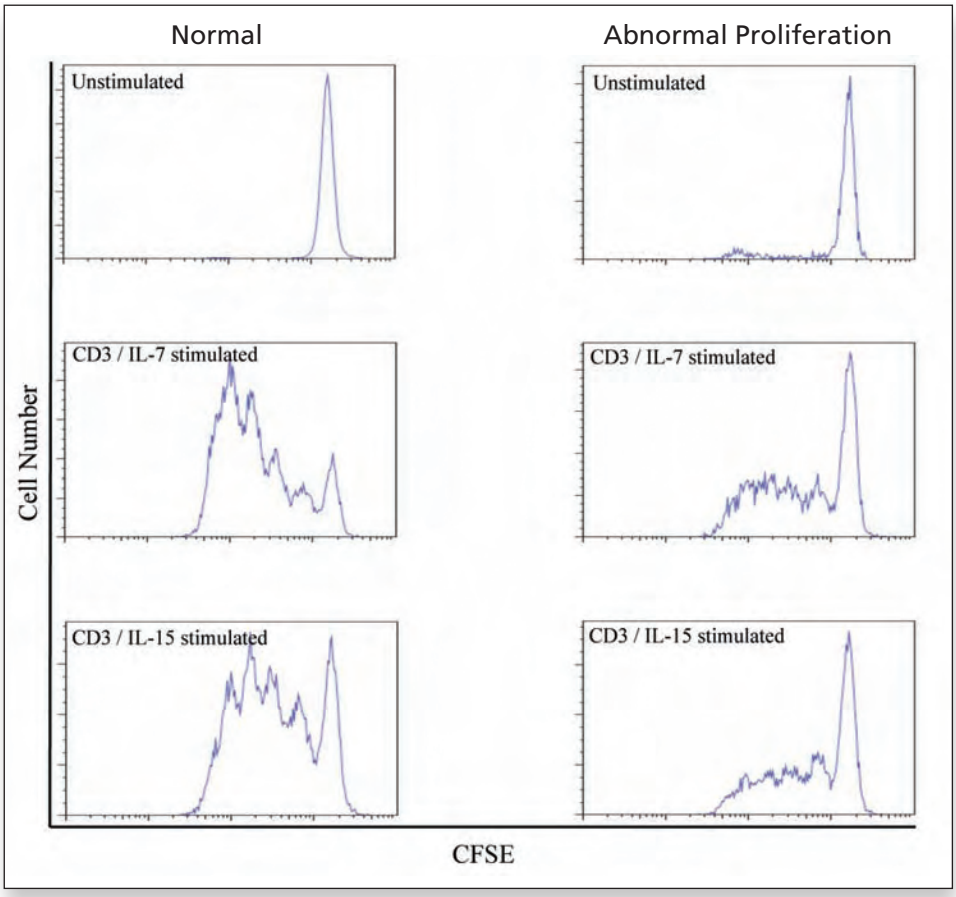
CPT codes: 86353 x 5, 88187.

Clinical indication/general description

Once a defect in T cells is detected, or defective T cell function is suspected, further evaluation involves examination of T cell proliferation in response to interleukins. Activation of T cells with antibodies to the T cell receptor and cytokines results in the proliferation of T cells over the next seven to 10 days. Diminished or absent proliferative response to T cell stimuli is consistent with a primary (such as severe combined immunodeficiency) or secondary immunodeficiency disease that affects T lymphocytes (cellular immunity). This assay tests the proliferative function of T cells in response to cytokines and a variety of growth factors to help determine if defective proliferation is the result of defective growth factor secretion or defective growth factor receptor expression. This assay is designed to be used only if the T mitogen assay is abnormal (see below).

Detection methodology

A functional flow cytometric-based assay provides a semi-quantitative assessment of lymphocyte proliferation to CD3/IL-2, CD3/IL-7, CD3/IL-15 and CD3/CD28. Lymphocytes are labeled with the fluorescent proliferation tracking dye CFSE and activated with cytokines. As lymphocytes divide, the fluorescent label is diluted in half, which can be seen on flow diagrams as peaks of decreasing fluorescence. Lymphocyte proliferative response is demonstrated by an increase in FSC/SSC and a progressive two-fold reduction in the proliferation tracking dye.



Toll-like Receptor

Utility: 1) Functional evaluation of toll–like receptors (TLRs). 2) Diagnostic screen for primary immunodeficiency related to the innate immune system.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

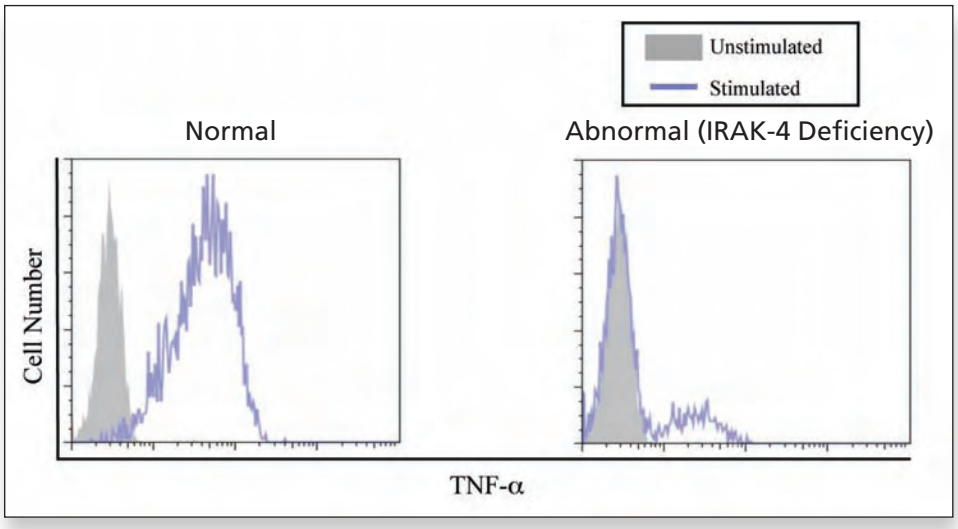
CPT codes: 86353 x 3, 88184, 88185, 81887.

Clinical indication/general description

TLRs recognize a variety of molecules conserved in microorganisms that are not present in humans, such as lipopolysaccharides in bacteria and double stranded RNA of viruses. Defects in signaling through TLR have been found in humans with increased susceptibility to infections. Defects in MyD88 and IRAK-4, molecules required for TLR4 signaling, have been detected in patients with recurrent, invasive pneumococcal or staphylococcal infections, and defects in TLR3 and UNC-93B have been found in patients with recurrent herpes encephalitis. Infants and young children are particularly susceptible to infections when they have defects in TLRs since the adaptive immune system has not developed at this age to offer protection. Any children with recurrent or severe pneumococcal, staphylococcal infections or herpes encephalitis should be evaluated for defective TLR signaling.

Detection methodology

A functional flow cytometric-based assay tests TLR4 function by assessing the ability of monocytes to produce tumor necrosis factor-alpha (TNF-α). Peripheral blood is incubated with lipopolysaccharide and TNF-α production is measured. This assay will detect defects in MyD88 and IRAK-4, proteins involved in TLR4 signaling that have been associated with recurrent, invasive pneumococcal and staphylococcal infections.



T cell Mitogen Proliferation

Utility: Functional evaluation of T cells proliferation to mitogens.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

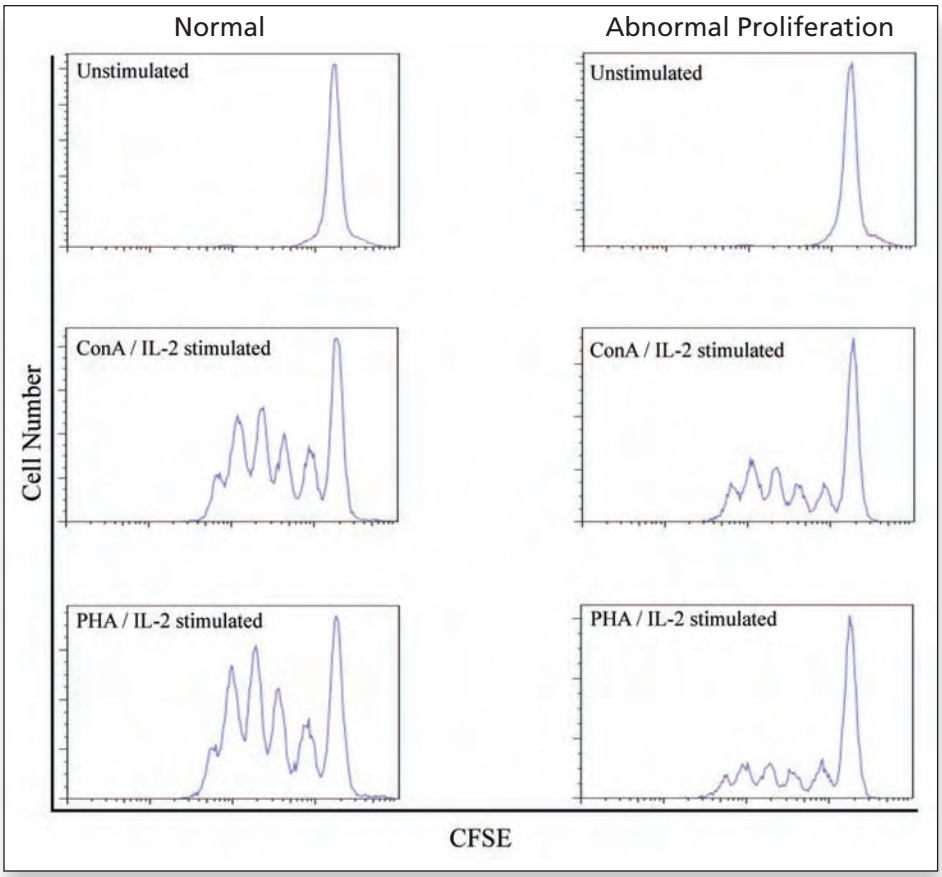
CPT codes: 86353 x 5, 88187.

Clinical indication/general description

Once a defect in T cells is detected, or defective T cell function is suspected, further evaluation involves examination of T cell proliferation in response to mitogens. Activation of T cells with antibodies to the T cell receptor or with plant lectins results in the proliferation of these cells over the next seven to 10 days. Diminished or absent proliferative response to T cell stimuli is consistent with a primary or secondary immunodeficiency disease that affects T lymphocytes (cellular immunity). This assay tests the proliferative function of T cells to T cell receptor antibodies and a variety of mitogens.

Detection methodology

A functional flow cytometric-based assay provides a semi-quantitative assessment of lymphocyte proliferation in response to concanavalin A (ConA)/IL-2, phytohemagglutinin (PHA)/IL-2, phorbol esters and soluble CD3. Lymphocytes are labeled with the fluorescent dye CFSE and activated with mitogens. As lymphocytes divide, the fluorescent label is diluted in half, which can be seen on flow diagrams as peaks of decreasing fluorescence. Lymphocyte proliferative response is demonstrated by an increase in FSC/SSC and a progressive two-fold reduction in a proliferation tracking dye. Lymphocyte proliferative response is demonstrated by an increase in FSC/SSC and a progressive two-fold reduction in a proliferation tracking dye.



T Regulatory – FOXP3 (TREG)

Utility: 1) Determine CD4+CD25+CD127dim+FoxP3+ regulatory T cells (Treg) in the peripheral blood. 2) Diagnostic screen for the presence of X-linked immunodysregulation, polyendocrinopathy and enteropathy (IPEX) by assessing T regulatory cells.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

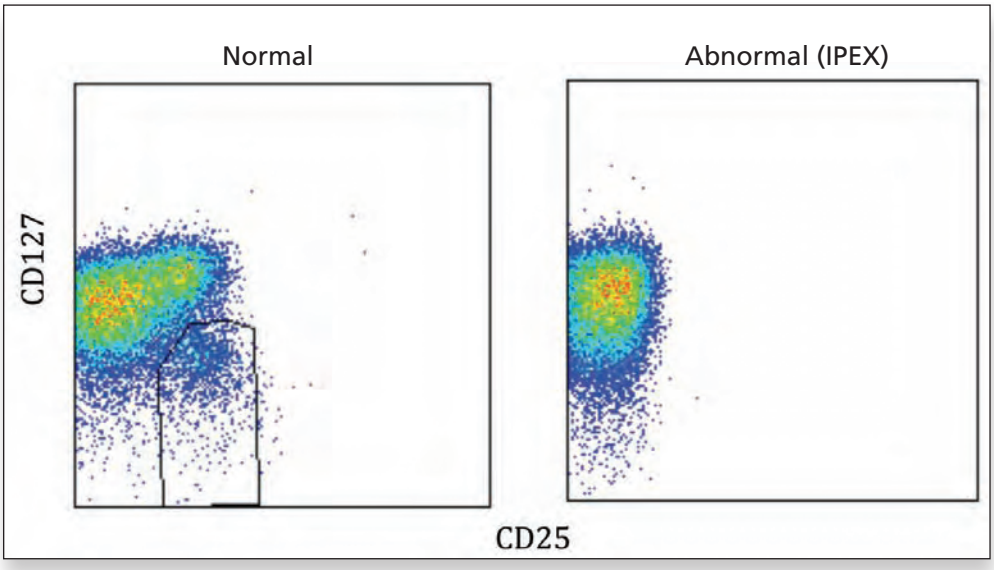
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 6, 88188.

Clinical indication/general description

IPEX is an X-linked recessive disorder causing widespread autoimmune manifestations. Mutations in the forkhead transcription factor *FoxP3* are responsible for this disease. FoxP3 is crucial to the development, survival and effector function of regulatory T (Treg) cells, a cell population essential to immune regulation. Treg cells account for approximately 5 to 10 percent of peripheral blood CD4+ T cells. Treg cells also express high levels of CD25, the high affinity binding alpha subunit of the IL-2 receptor, and low levels of CD127, the IL-7 receptor. IPEX-like diseases also can be caused by deficiencies in CD25 or STAT5b, key signal transduction subunits of the IL-2 receptor.

Detection methodology

Use of antibodies to CD4, CD25, CD127 and FoxP3 allows for the determination of the percentage of Treg cells in peripheral blood of patients suspected of IPEX or IPEX-like diseases.



X-linked Lymphoproliferative Syndrome (XLP)

Utility: 1) Evaluate the presence of SLAM-associated protein (SAP) and X-linked inhibitor of apoptosis (XIAP) in peripheral blood. 2) Diagnostic screen for X-Linked Lymphoproliferative Syndrome (XLP), types 1 and 2. 3) Detection of carrier status in female relatives of XLP patients.

Specimen: 8 – 15 mL peripheral blood in sodium heparin (green top).

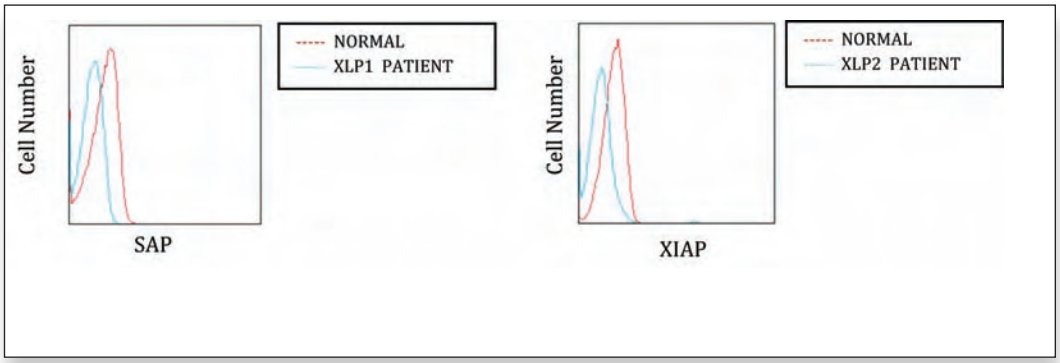
CPT codes: 86359, 86360, 86355, 86357, 88184, 88185 x 15, 88188.

Clinical indication/general description

XLP, also called Duncan disease, is a rare X-linked disorder affecting predominantly male patients. Mutations in *SH2D1A* gene encoding the signaling lymphocyte activation molecule (SLAM)-associated protein (SAP) cause XLP1. XLP type 2 (XLP2) is caused by mutations in *BIRC4* gene encoding the XIAP protein.

Detection methodology

Intracellular protein expression of SAP and XIAP in peripheral blood lymphocytes is determined by flow cytometry. Deficient expression of SAP and XIAP in lymphocytes is associated with XLP type 1 or XLP type 2, respectively.



Test name	Code	Diagnostic utility
Autoimmune Lymphoproliferative Syndrome	AILYMP	Screen for ALPS.
Absolute T4	AT4	Monitor treatment efficacy for HIV.
Bruton's Tyrosine Kinase	BTK	• Screen for X-linked agammaglobulinemia. • Carrier status detection in female relative of XLA.
Common Variable Immunodeficiency	CVID	Screen for CVID.
Cytotoxicity/Apoptosis	CYTAPO	• Screen for hemophagocytic lymphohistiocytosis. • Functional evaluation of natural killer function.
Hyper IGM	HIGM	• Screen for X-linked (CD40L) Hyper IgM. • Screen for autosomal recessive (CD40) Hyper IgM. • Carrier status detection in female relative of XL-HIGM.
Neutrophil Oxidative Burst	NEUOXB	• Functional evaluation of neutrophil oxidative burst. • Screen for chronic granulomatous disease. • Carrier status detection in female relative of CGD.
Neutrophil Phagocytosis	PHAGO	Functional evaluation of Neutrophil Phagocytosis.
Perforin-Granzyme	PERGRA	• Screen for hemophagocytic lymphohistiocytosis. • Perforin, granzyme A, granzyme B detection in lymphocytes.

Test name	Code	Diagnostic utility
Primary Immunodeficiency 1	PID 1	General T, B and NK evaluation of immune status.
Primary Immunodeficiency 2	PID 2	• Comprehensive screen for cellular and humoral immunity. • Screen for leukocyte adhesion deficiency I and II.
Severe Combined Immunodeficiency	SCID	Confirmatory test of SCID in newborns.
T cell Activation	TCACT	Functional evaluation of T cell activation.
T helper IL17	THIL17	Screen for hyper-immunoglobulin E syndrome.
T cell Interleukin Proliferation	TINTL	Functional evaluation of T cell proliferation to interleukins.
Toll-like Receptor	TLREC	• Functional evaluation of toll-like receptor. • Screen for innate immune system immuno-deficiencies.
T cell Mitogen Proliferation	TMITO	Functional evaluation of T cell proliferation to mitogens.
T Regulatory – FOXP3	TREG	Diagnostic screen for the presence of X-linked Immunodysregulation, Polyendocrinopathy and Enteropathy (IPEX) by assessing T regulatory cells.
X-Linked Lymphoproliferative Syndrome	XLP	Diagnostic screen for X-linked lymphoproliferative syndrome, type 1 (XLP1) and type 2 (XLP2) by assessing SAP and XIAP proteins expression.

Primary immunodeficiency diagnosis and treatment

While identification of primary immunodeficiency diseases can be difficult, timely diagnosis and treatment prevents complications and saves lives. We can help.

Clinical Immunodiagnostic and Research Laboratory

The Medical College of Wisconsin

Our lab:

- Offers a comprehensive array of tests to aid in diagnosis of PID and other hematopoietic disorders.
- Technical staff members are certified medical laboratory scientists with special expertise in use of flow cytometric methodologies.
- Participates in proficiency programs offered by the College of American Pathologists and meets all requirements of the Clinical Laboratory Improvement Amendments.

For information about testing services
Trivikram Dasu, PhD, laboratory manager
(414) 456-4165
tdasu@mcw.edu
mcw.edu/CIRL (includes a link to the lab request form)

Note: Specimen requirements vary depending on the test type, patient's WBC count, absolute lymphocytes count, the number of immature or abnormal cells present and the patient's history.

Specimen delivery address
Clinical Immunodiagnostic and Research Laboratory
The Medical College of Wisconsin
MACC Fund Research Center
8701 Watertown Plank Road, Room 5072
Milwaukee, WI 53226

Primary immunodeficiency diagnosis and treatment

Children's Hospital of Wisconsin

Our clinic:

- Evaluates children and adults and offers a full range of treatments.
- Provides multidisciplinary care from experts in Allergy and Clinical Immunology, Rheumatology, Hematology, Oncology, Blood and Marrow Transplant and Infectious Disease.
- Conducts research to improve diagnostic testing and care.

For information about our program
chw.org/pip

To refer a patient or consult with a physician
Mary Hintermeyer, MSN, RN, advanced practice nurse practitioner
(414) 266-6293
mhintermeyer@chw.org
Allergy/Immunology Administrative Offices – (414) 266-6840

Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiency

John Routes, MD
Program Leader
Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiency
Professor of Pediatrics and Chief, Section of Allergy and Clinical Immunology
The Medical College of Wisconsin
Medical Director, Allergy/Clinical Immunology
Children's Hospital of Wisconsin

James Verbsky, MD, PhD
Associate professor of Pediatrics, Section of Pediatric Rheumatology
Director, Clinical Immunodiagnostic Research Laboratory
The Medical College of Wisconsin
Pediatric Rheumatology
Children's Hospital of Wisconsin

James Casper, MD
Professor of Pediatrics, Section of Hematology/Oncology
The Medical College of Wisconsin
Pediatric Hematology/Oncology
Children's Hospital of Wisconsin

Our program is a nationally recognized referral center and one of only 14 Jeffrey Modell Foundation Diagnostic and Research Centers of Excellence in the country.

Our physicians worked with the Wisconsin State Laboratory of Hygiene to make Wisconsin the first state to pilot a newborn screening test for severe combined immunodeficiency. The test identifies SCID early enough to help treat children impacted by this often fatal disease.

Exacta 100770 7,700 mlk 0411

Children's Hospital of Wisconsin®

A member of Children's Hospital and Health System.

Children's Hospital of Wisconsin, Inc.
PO Box 1997
Milwaukee, WI 53201-1997